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LETTER TO THE EDITOR

Prevalence and Molecular Characterization of *Escherichia coli* Clinical Isolates Carrying *mcr-1* in a Chinese Teaching Hospital from 2002 to 2016

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Colistin will be gradually banned from animal feeds in China and switched to clinical human therapy in the near future (1). However, the presence of the transferable colistin resistance gene *mcr-1* in *Escherichia coli* clinical isolates in Chinese hospitals is still poorly understood. In this study, we aimed to investigate and shed light on the prevalence and molecular characteristics of *mcr-1*-positive *E. coli* clinical isolates in a Chinese teaching hospital from 2002 to 2016.

A collection of 3,434 *E. coli* clinical isolates collected at the First Affiliated Hospital of Wenzhou Medical University in China from 2002 to 2016 were screened for the presence of *mcr-1* using PCR. Twelve *mcr-1*-positive *E. coli* isolates (0.35% [12/3,434]) were detected during this 15-year period, with the first *mcr-1*-positive *E. coli* isolate identified in our hospital in 2010. The other *mcr-1*-positive *E. coli* isolates were isolated in 2012 (1 isolate), 2015 (7 isolates), and 2016 (3 isolates) (Table 1). Antimicrobial susceptibility testing conducted using the broth microdilution method revealed that all 12 *mcr-1*-positive *E. coli* isolates exhibited resistance to colistin, third-generation cephalosporins ($bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$), and quinolone antibiotics [*aac*(6′)-*Ib-cr* and *qnrA*, with mutations in GyrA and ParC]. Furthermore, isolates DC2562 and DC90 were resistant to carbapenems, while DC3539, DC3599, DC3802, DC3806, DC3846, DC4887, and DC5262 were resistant to aminoglycosides associated with *aac*(6′)-*Ib-cr* (Table 2). The results of PCR and sequencing showed most of the *mcr-1*-positive *E. coli* isolates carried two extended-spectrum β-lactamase (ESBL) genes and/or quinolone resistance genes; DC2562 and DC90 also carried carbapenem resistance gene $bla_{OXA\text{-}48}$, which was detected by hybridization on the same IncI1 plasmid as *mcr-1* in strain DC2562 (Table 2). However, these patients, who were suffering from infections, were not treated with colistin therapy during hospitalization.

Conjugation experiments showed that 10 out of 12 *mcr-1*-positive *E. coli* isolates were able to successfully transfer *mcr-1* to the recipient strain *E. coli* EC600. The MICs of colistin for these 10 transconjugants were either 4 or 8 μg/ml, and they were also resistant to cephalosporins and/or carbapenems. The results of PCR and sequencing further revealed that transconjugants harbored *mcr-1* and β-lactamase resistance genes (Table 2) (2). S1 pulsed-field gel electrophoresis (PFGE) and Southern blotting also confirmed that 10 out of 12 of the *mcr-1*-positive *E. coli* isolates carried *mcr-1* on two ~33- or ~62-kb plasmids (see Fig. S1 in the supplemental material). For the other two *mcr-1*-positive *E. coli* isolates (DC3411 and DC3806), no plasmid localization could be evidenced (3). Replicon typing successfully identified 10 transconjugants that carried

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TABLE 1 Clinical characteristics and MLSTs of 12 *mcr-1*-positive *E. coli* isolates

Isolate	Date (mo/yr)	Specimen	Gender	Age range (yr)	Outcome	Hospital stay (days)	Clinical diagnosis[a]	MLST type
DC2562	11/2010	Blood	Female	40–49	Discharged	30	Urinary tract infection, polycystic kidney syndrome	ST44
DC90	3/2012	Pus	Male	80–89	Discharged	50	Urinary tract infection, pulmonary infection, ileus, MODS	ST2
DC3411	2/2015	Urine	Male	70–79	Improvement	14	Bladder tumors	ST2
DC3539	3/2015	Drainage fluid	Male	80–89	Discharged	14	Sigmoid tumor, chronic bronchitis	ST730
DC3599	3/2015	Sputum	Male	70–79	Improvement	36	AECOPD, pulmonary infection	ST45
DC3658	4/2015	Blood	Male	70–79	Improvement	19	Ischemic stroke, pulmonary infection, urinary tract infection	ST48
DC3802	5/2015	Wound	Female	60–69	Discharged	10	Chronic skin ulcer	ST1
DC3806	5/2015	Sputum	Female	10–19	Improvement	54	NHL, pulmonary infection	ST31
DC3846	5/2015	Urine	Female	70–79	Discharged	16	Cystitis, urinary tract infection	ST632
DC4887	2/2016	Urine	Male	60–69	Discharged	7	Indirect inguinal hernia, urinary tract infection	ST53
DC5262	5/2016	Urine	Female	30–39	Improvement	36	Acute liver failure, urinary tract infection	New[b]
DC5286	5/2016	Urine	Female	80–89	Improvement	7	Three-degree atrioventricular block	ST506

[a]MODS, multiple organ dysfunction syndrome; AECOPD, acute exacerbations of chronic obstructive pulmonary disease; NHL, non-Hodgkin lymphoma.
[b]One novel ST that is currently not registered in the MLST database.

mcr-1 plasmids obtained from 12 *mcr-1*-positive *E. coli* isolates and belonged to five Inc groups: IncI1, IncP, IncFIB, IncI1, and IncW type (Table 2).

PFGE analysis showed that these isolates harboring *mcr-1* were clonally unrelated, except DC90 and DC3411. Multilocus sequence typing (MLST) analysis further assigned the isolates to 10 distinct sequence types (STs), of which eight of the *mcr-1*-positive *E. coli* isolates were first reported, along with an additional novel ST (currently not registered in the MLST database) (Table 1).

TABLE 2 MICs, resistance genes, and plasmid profiles of *mcr-1*-positive *E. coli* isolates

Isolate	MIC (μg/ml)[a]												*mcr-1* plasmid (size in kb)	Other resistance genes[b]
	CAZ	CTX	MEM	IPM	ETP	AMP	GEN	AMK	CIP	LVX	NIT	CST		
DC2562	≥64	≥32	1	2	4	≥32	4	2	>16	16	32	8	IncI1 (62)	$bla_{CTX-M-1}$, bla_{OXA-48},* *aac(6′)-Ib-cr*, *qnrA*, *gyrA*(*S83L*), *parC*(*S80I*)
DC90	≥64	≥32	1	2	2	≥32	2	2	>16	>16	64	8	IncP (33)	$bla_{CTX-M-9}$,* bla_{OXA-48}, *aac(6′)-Ib-cr*, *qnrA*, *parC*(*S80I G144V*), *gyrA*(*S83L*)
DC3411	16	≥32	0.015	0.125	0.5	≥32	2	2	>16	16	16	4	ND[c]	$bla_{CTX-M-9}$, bla_{TEM}, *aac(6′)-Ib-cr*, *qnrA*
DC3539	32	≥32	0.015	0.125	0.5	≥32	>64	16	>16	16	64	16	IncI1 (62)	$bla_{CTX-M-1}$,* $bla_{CTX-M-9}$, *aac(6′)-Ib-cr*, *qnrA*
DC3599	32	≥32	0.03	0.06	0.5	≥32	64	16	>16	16	64	8	IncI1 (62)	$bla_{CTX-M-1}$, $bla_{CTX-M-9}$,* *aac(6′)-Ib-cr*, *qnrA*
DC3658	32	≥32	0.015	0.125	0.5	≥32	2	4	16	4	64	8	IncW (62)	$bla_{CTX-M-1}$, $bla_{CTX-M-9}$,* *aac(6′)-Ib-cr*, *qnrA*, *qnrD*
DC3802	≥64	≥32	0.03	0.125	0.5	≥32	>64	2	>16	16	8	4	IncFIB (62)	$bla_{CTX-M-1}$, $bla_{CTX-M-9}$,* *aac(6′)-Ib-cr*, *gyrA*(*S83L*)
DC3806	≥64	≥32	0.03	0.125	0.5	≥32	>64	2	>16	>16	32	8	ND	$bla_{CTX-M-1}$, $bla_{CTX-M-9}$, *aac(6′)-Ib-cr*, *qnrA*
DC3846	≥64	≥32	0.03	0.5	0.5	≥32	>64	4	>16	>16	64	16	IncFIB (62)	$bla_{CTX-M-1}$, $bla_{CTX-M-9}$,* *aac(6′)-Ib-cr*, *qnrA*, *qnrD*
DC4887	16	≥32	0.015	0.125	0.5	4	>64	2	>16	8	16	8	IncFIB (62)	$bla_{CTX-M-1}$,* *aac(6′)-Ib-cr*, *gyrA*(*S83L*)
DC5262	≥64	≥32	1	1	0.5	4	16	8	0.5	1	16	8	IncFIB (62)	bla_{TEM-1},* *aac(6′)-Ib-cr*, *parC*(*S80I*)
DC5286	≥64	≥32	0.03	0.125	0.5	2	2	4	>16	16	16	8	IncFIB (62)	$bla_{CTX-M-1}$,* *aac(6′)-Ib-cr*, *gyrA*(*S83L*)
EC600	0.5	2	0.03	0.125	0.06	4	2	2	0.25	0.5	8	0.5	ND	ND

[a]CAZ, ceftazidime; CTX, cefotaxime; MEM, meropenem; IPM, imipenem; ETP, ertapenem; AMP, ampicillin; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; LVX, levofloxacin; NIT, nitrofurantoin; CST, colistin.
[b]Asterisks represent the genes that were coidentified in the transconjugants.
[c]ND, not detected.

In summary, the *mcr-1*-positive *E. coli* isolate in our study was first isolated in 2010, highlighting an earlier existence of *mcr-1* in clinical patients in mainland China than previously reported (4–7). Although a low prevalence of *mcr-1* was determined in *E. coli* clinical isolates in China, further monitoring and management of the prevalence of *mcr-1* in clinical isolates are urgently needed.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02623-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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REFERENCES

1. Wang Y, Tian GB, Zhang R, Shen Y, Tyrrell JM, Huang X, Zhou H, Lei L, Li HY, Doi Y, Fang Y, Ren H, Zhong LL, Shen Z, Zeng KJ, Wang S, Liu JH, Wu C, Walsh TR, Shen J. 2017. Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect Dis 17:390–399. https://doi.org/10.1016/S1473-3099(16)30527-8.
2. Zhong LL, Zhang YF, Doi Y, Huang X, Zhang XF, Zeng KJ, Shen C, Patil S, Xing Y, Zou Y. 2017. Coproduction of MCR-1 and NDM-1 by colistin-resistant *Escherichia coli* isolated from a healthy individual. Antimicrob Agents Chemother 61:e01962-16. https://doi.org/10.1128/AAC.01962-16.
3. Yu H, Qu F, Shan B, Huang B, Jia W, Chen C, Li A, Miao M, Zhang X, Bao C, Xu Y, Chavda KD, Tang YW, Kreiswirth BN, Du H, Chen L. 2016. Detection of the *mcr-1* colistin resistance gene in carbapenem-resistant *Enterobacteriaceae* from different hospitals in China. Antimicrob Agents Chemother 60:5033–5035. https://doi.org/10.1128/AAC.00440-16.
4. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16:161–168. https://doi.org/10.1016/S1473-3099(15)00424-7.
5. Quan J, Li X, Chen Y, Jiang Y, Zhou Z, Zhang H, Sun L, Ruan Z, Feng Y, Akova M, Yu Y. 2017. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. Lancet Infect Dis 17:400–410. https://doi.org/10.1016/S1473-3099(16)30528-X.
6. Fernandes MR, Moura Q, Sartori L, Silva KC, Cunha MP, Esposito F, Lopes R, Otutumi LK, Goncalves DD, Dropa M, Matte MH, Monte DF, Landgraf M, Francisco GR, Bueno MF, de Oliveira Garcia D, Knobl T, Moreno AM, Lincopan N. 2016. Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene. Euro Surveill https://doi.org/10.2807/1560-7917.ES.2016.21.17.30214.
7. Saavedra SY, Diaz L, Wiesner M, Correa A, Arevalo SA, Reyes J, Hidalgo AM, de la Cadena E, Perenguez M, Montano LA, Ardila J, Rios R, Ovalle MV, Diaz P, Porras P, Villegas MV, Arias CA, Beltran M, Duarte C. 2017. Genomic and molecular characterization of clinical isolates of *Enterobacteriaceae* harboring *mcr-1* in Colombia, 2002 to 2016. Antimicrob Agents Chemother 61:e00841-17. https://doi.org/10.1128/AAC.00841-17.